# Plasma proendothelin-1 as an early marker of bronchopulmonary dysplasia

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Abstract: BACKGROUND: Bronchopulmonary dysplasia (BPD) is a common complication in preterm infants. Clinical prediction of BPD at an early stage in life is difficult. Plasma proendothelin-1 (CT-proET-1) is a lung injury biomarker in pulmonary hypertension and respiratory distress. OBJECTIVE: To assess the prognostic ability of CT-proET-1 in BPD. METHODS: In 227 prospectively enrolled preterm infants born at <32 weeks gestational age (GA), plasma CT-proET-1 was measured at birth, day of life (DOL) 2, 3, 6 and 28, and at 36 weeks postmenstrual age (PMA). BPD was defined as mild in infants requiring supplemental oxygen at DOL 28 and moderate/severe in those requiring it at 36 weeks PMA. RESULTS: The predictive ability of CT-proET-1 for any BPD was poor at birth [area under the ROC curve (AUC) 0.654, 95% CI 0.494-0.814], moderate at DOL 2 and 3 (AUC 0.769, 95% CI 0.666-0.872) and excellent at DOL 6 (AUC 0.918, 95% CI 0.840-0.995). Multivariable regression analysis revealed that CT-proET-1 levels at DOL 2, 3, 6 and 28 were strongly related to the duration of oxygen supplementation, independently of GA and the duration of respiratory support. CONCLUSIONS: CT-proET-1 is a novel promising biomarker for predicting the development of BPD in preterm infants when measured at the end of the first week of life.

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# Plasma Proendothelin-1 as an Early Marker of Bronchopulmonary Dysplasia

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**Key Words**
Chronic lung disease · Prematurity · Neonate · Biomarker · Vasoactive peptide

**Abstract**
***Background:*** Bronchopulmonary dysplasia (BPD) is a common complication in preterm infants. Clinical prediction of BPD at an early stage in life is difficult. Plasma proendothelin-1 (CT-proET-1) is a lung injury biomarker in pulmonary hypertension and respiratory distress. ***Objective:*** To assess the prognostic ability of CT-proET-1 in BPD. ***Methods:*** In 227 prospectively enrolled preterm infants born at <32 weeks gestational age (GA), plasma CT-proET-1 was measured at birth, day of life (DOL) 2, 3, 6 and 28, and at 36 weeks postmenstrual age (PMA). BPD was defined as mild in infants requiring supplemental oxygen at DOL 28 and moderate/severe in those requiring it at 36 weeks PMA. ***Results:*** The predictive ability of CT-proET-1 for any BPD was poor at birth [area under the ROC curve (AUC) 0.654, 95% CI 0.494–0.814], moderate at DOL 2 and 3 (AUC 0.769, 95% CI 0.666–0.872) and excellent at DOL 6 (AUC 0.918, 95% CI 0.840–0.995). Multivariable regression analysis revealed that CT-proET-1 levels at DOL 2, 3, 6 and 28 were strongly related to the duration of oxygen supplementation, independently of GA and the duration of respiratory support. ***Conclusions:*** CT-proET-1 is a novel promising biomarker for predicting the development of BPD in preterm infants when measured at the end of the first week of life.


## Introduction

Bronchopulmonary dysplasia (BPD) is a common complication of premature birth with important long-term consequences for the increasing number of very preterm infants who survive [1]. Endothelin-1 (ET-1) is an endogenous vasoconstrictive peptide, which is involved in the pathogenesis of various lung disorders such as pulmonary hypertension, acute respiratory failure and pulmonary fibrosis [2]. In preterm newborns, raised plasma ET-1 levels have been related to the development of respiratory distress syndrome [3] whereas an

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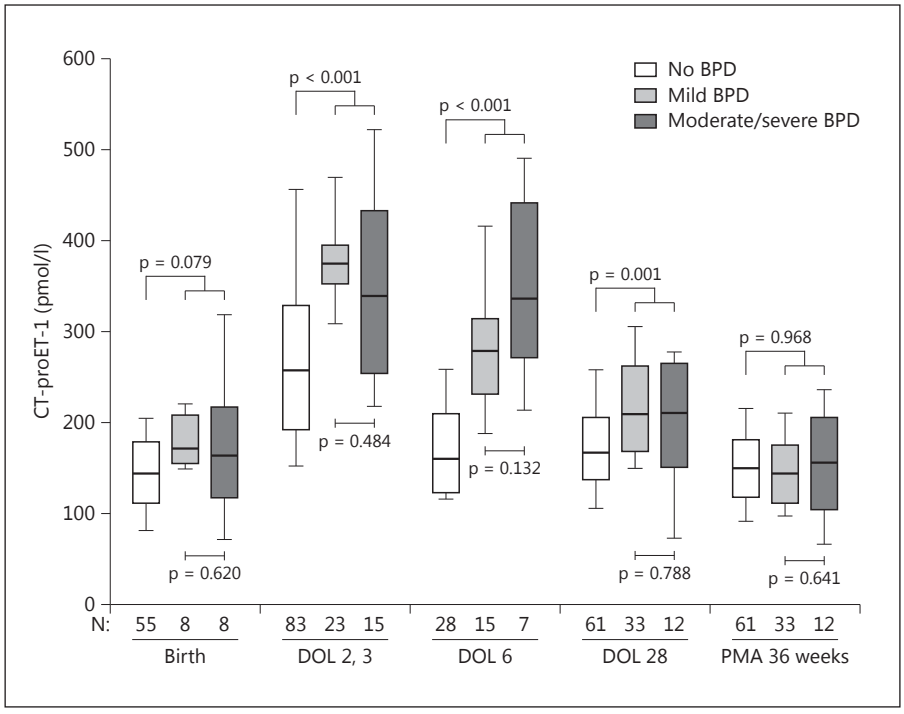


**Fig. 1.** Circulating CT-proET-1 levels at different postnatal time points with respect to the severity of BPD. Between-group comparisons were performed with the Mann-Whitney U test. The number of measurements (N) at each time point is also noted. Data do not represent longitudinal measurements. Plasma CT-proET-1 at DOL 2 and 6 derives from 2 separate cohorts (A and B) [8, 9], while values at DOL 2–3 represent the combination of CT-proET-1 measurements from these 2 cohorts. Circulating CT-proET-1 measurements at DOL 28 and at 36 weeks PMA derived from a third cohort (C).

early increase of ET-1 in the tracheal aspirate has been shown to correlate with subsequent progression to BPD [4]. In addition, the antiangiogenic effect of ET-1 has been linked to the pathogenesis of BPD [5] and evidence from human ex vivo models suggests that ET-1 signaling may play a critical role in the development of a BPD-like fibrotic process in the immature lung [6]. Although ET-1 is unstable in the peripheral blood and therefore less suited for diagnostic use, the more stable C-terminal fragment of the ET-1 precursor (CT-proET-1) can be used to estimate ET-1 release [7]. Our group has recently reported that plasma CT-proET-1 levels at day of life (DOL) 3 are strongly correlated with the development and severity of respiratory distress syndrome in preterm newborns [8]. However, the prognostic ability of ET-1 or CT-proET-1 in relation to BPD remains unknown.

## Subjects and Methods

This study was based on prospectively collected data and blood samples from 227 neonates born at a gestational age (GA) of <32 weeks and cared for at the University Hospitals of Basel and Zurich, Switzerland. A fraction of the participants (n = 121) had been enrolled in 2 previous studies aiming to explore plasma CT-proET-1 levels in neonates in relation to respiratory morbidity at birth with measurements at birth and DOL 3 (n = 71, cohort A) [8] and in the context of patent ductus arteriosus with measurements at DOL 2 and 6 (n = 50, cohort B) [9]. The remaining 106 participants were newly enrolled and had CT-proET-1 measurements at DOL 28 and at a postmenstrual age (PMA) of 36 weeks (cohort C, Clinical Trial Registration NCT01644981). The study was approved by the institutional review boards of both university hospitals. Written informed consent was obtained from the parents prior to enrollment.

A blood volume of 500 μl was collected in EDTA microtubes simultaneously with routine blood sampling at different postnatal time points (i.e. at birth, DOL 2, 3, 6 and 28, and at 36 weeks PMA) depending on the study cohort as was analyzed above. Samples were immediately transferred to the central laboratory of both study sites for centrifugation and storage at −20°C until analysis. CT-proET-1 levels were measured in batches at the Institute of Clinical Chemistry of the University Hospital Zurich using a fully automated immunofluorescent assay (KRYPTOR, BRAHMS Biomarkers, Thermo Fisher Scientific, Hennigsdorf, Germany). CT-proET-1 assay precision was for repeated serial measurements 1.6% at 229.9 pmol/l (intraday, n = 10) and for day-to-day measurements 4.1% at 226.3 pmol/l (interday, n = 10). The laboratory staff was blinded to the aims of the study. BPD was defined as mild in infants requiring supplemental oxygen at DOL 28 and moderate/severe in those requiring supplemental oxygen at 36 weeks PMA. Since there were no differences in CT-proET-1 values at DOL 2 and 3 between cohorts A and B, respectively (online suppl. fig.; for all suppl. material, see www.karger.com/doi/10.1159/000438979), these measurements were combined into 1 group (i.e. CT-proET-1 values at DOL 2 and 3). Plasma CT-proET-1 levels were compared (Mann-Whitney U

**Table 1.** Predictors of the duration of oxygen dependency

| Model: | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| $R^2$: | 0.431 | 0.471 | 0.526 | 0.447 | 0.411 |
| GA | −0.355[b] | −0.313[c] | −0.107 | −0.415[c] | −0.285 |
| Days of respiratory support[a] | 0.307 | 0.301[c] | 0.116 | 0.370[c] | 0.389[b] |
| CT-proET-1 | | | | | |
| Birth | 0.154 | – | – | – | – |
| DOL 2 | – | 0.259[b] | – | – | – |
| DOL 6 | – | – | 0.548[d] | – | – |
| DOL 28 | – | – | – | 0.226[b] | – |
| PMA 36 weeks | – | – | – | – | −0.049 |

Multivariable regression model using the log-transformed days of oxygen requirement as the dependent variable. Data represent adjusted regression β coefficients.

[a] Days of mechanical ventilation and/or nasal continuous positive airway pressure.

[b] $p < 0.05$; [c] $p < 0.01$; [d] $p < 0.001$.

test) between infants with and without BPD at birth (umbilical cord blood), DOL 2, 3, 6 and 28, and at 36 weeks PMA. Multivariable logistic regression models were used to explore the relation between CT-proET-1 levels at the above-mentioned time points as well as the duration of oxygen dependency (after logarithmic transformation).

## Results

The characteristics of the three cohorts are presented in the online supplementary table. The overall prevalence of BPD was 36.6% (n = 83), while 27 infants (11.9%) had moderate/severe disease. Circulating CT-proET-1 levels at birth and at 36 weeks PMA did not differ between infants with and without BPD (fig. 1). Conversely, plasma CT-proET-1 levels at DOL 2, 3, 6 and 28 were significantly higher in infants with BPD compared to non-BPD subjects [median (IQR): 372 (319–409) vs. 258 (192–329) pmol/l at DOL 2 and 3, 291 (240–340) vs. 160 (123–210) pmol/l at DOL 6 and 210 (169–264) vs. 167 (137–206) pmol/l at DOL 28]. No differences were found between infants with mild and moderate/severe BPD at any postnatal time point, but at DOL 6, there was a trend towards higher CT-proET-1 values in infants who later developed severe/moderate disease. The predictive ability of plasma CT-proET-1 was poor at birth [area under the ROC curve (AUC) 0.654, 95% CI 0.494–0.814], moderate at DOL 2 and 3 (AUC 0.769, 95% CI 0.666–0.872) and excellent at DOL 6 (AUC 0.918, 95% CI 0.840–0.995). Multivariable regression analysis revealed that CT-proET-1 levels at DOL 2 3, 6 and 28 were strongly related to the duration of oxygen supplementation independent of GA and the duration of respiratory support (mechanical ventilation and/or nasal continuous positive airway pressure; table 1). Interestingly, at DOL 6, the effect of CT-proET-1 was so strong that it overwhelmed the effect of the other confounders.

## Discussion

Our findings suggest that CT-proET-1 may be a promising biomarker for predicting the development of BPD in preterm neonates. This is not surprising, since ET-1 signaling has been shown to be involved in the disruption of molecular pathways that promote alveolar development and repair, especially in the immature lung [6, 10]. Given our reported time-specific differences in plasma CT-proET-1 levels between neonates who did or did not develop BPD, we propose that CT-proET-1 measurements at the end of the first week of life might be of maximum diagnostic accuracy. Such an early identification of infants at risk of BPD would be of paramount clinical importance, because it could allow for the design, implementation, and evaluation of personalized preventive and therapeutic strategies [11]. The main limitation of our study is that it was based on CT-proET-1 measurements which derived in a cross-sectional manner from 3 independent cohorts and at different postnatal time points. Therefore, our findings about the relation between CT-proET-1 and BPD are suggestive but not conclusive. A sufficiently powered prospective trial of serial plasma CT-proET-1 measurements is required to further evaluate the usefulness of CT-proET-1 in quantifying lung injury and predicting progression to BPD in very preterm neonates.


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## Disclosure Statement

The authors have no conflicts of interest to disclose.

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